ET-42. DEVELOPMENT OF A NOVEL, FUNCTION-BLOCKING ANTI-FIBULIN-3 ANTIBODY AS TARGETED REAGENT FOR Glioblastoma

Mohan Nandhu1,2, Prajna Behera1,2, E. Antonio Chiocca1,2, and Mariano Viapiano1,2; 1Brigham and Women’s Hospital, Boston, MA, USA; 2Harvard Medical School, Boston, MA, USA

Fibulin-3 is a protein secreted by glioblastoma (GBM) cells that is virtually absent in normal brain. This protein promotes tumor invasion and, more unusually, apoptotic resistance, by paracrine activation of Notch and NFkB pathways (see companion abstract). Conditional knockdown of fibulin-3 reduces GBM growth, invasion and vascularization, and prolongs survival. Here we report initial validation of biologics generated against this unique target in GBM. We first identified the sequence of fibulin-3 that activates Notch signaling (aa25-47) and developed an immunizing peptide maintaining a required Cys-loop within this sequence. An IgG1k monoclonal antibody generated against this peptide (mAb428.2) showed high affinity for fibulin-3 (Kd 5 nM) and no cross-reactivity against highly homologous fibulin-4 or -5. mAb428.2 detected fibulin-3 in the stroma and capillaries of human GBM without cross-reactivity against normal brain. The antibody (50-250 μg/ml) blocked the activation of Notch and NFkB reporters induced by fibulin-3, and caused significant GBM cell cytotoxicity but had no effects on astrocytes or HEK293 cells, which do not express fibulin-3. IV injection of mAb428.2 (range: 0.1-30 mg/kg, evaluation: 15min to 14 days) did not cause adverse effects and showed no histological findings. Using Alexa750-labeled mAb428.2 (IV) we observed rapid elimination through urine and slow accumulation in liver and lungs. Therefore, we performed initial efficacy tests with a GBM stem cell model implanted subcutaneously. mAb428.2 (4 x 10 mg/kg) or control IgG1 were administered intratumorally and animals sacrificed at 1 days. Results indicated 45% median volume reduction in mAb428.2-injected tumors, with significant tumor necrosis. To further develop targeted reagents against fibulin-3 we have subcloned the Vh and Vl sequences of mAb428.2 and already generated a single-chain variable fragment (scFv) that recognizes fibulin-3. Our overall encouraging results suggest that fibulin-3 is a novel target with high potential for developing targeted agents against GBM.